drawn recently to experimental noise as a source of false-positive outcomes (Schnabel, 2008). It is possible that the Cerletti et al. (2008) study may, likewise, have been impacted by the problem of experimental noise.

Supplemental Data

Supplemental Data include one figure and can be found with this article online at http://www.cell.com/supplemental/S0092-8674(08)01508-0.

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Response

Skeletal Muscle Precursor Grafts in Dystrophic Mice

In his Correspondence, Terence Partridge raises two issues regarding our recent study investigating the stem cell properties of FACS-purified skeletal muscle precursors (SMPs) (Cerletti et al., 2008). First, he challenges our conclusion that SMPs represent a distinct myogenic cell population, and second, he questions our physiological assessment of the SMP-engrafted muscles of dystrophic *mdx* mice. However, as elaborated below, his point regarding the novelty of our cell isolation strategy is inaccurate, and more importantly, his reanalysis of our functional data uses inappropriate statistical methods that lead him to an erroneous conclusion.

First, Dr. Partridge argues that SMPs are not a unique myogenic cell population because (1) SMPs share phenotypic markers with muscle satellite cells, and (2) a previous publication (Montarras et al., 2005), which he coauthored, isolated a similar population of cells using gene-targeted Pax3-GFP reporter mice and another cell-surface marker (CD34 expression).

We agree that SMPs have properties similar to Pax3-GFP$^+$ muscle satellite cells and properly credit this work in our paper (Cerletti et al., 2008, p. 42). However, Dr. Partridge seems to disregard direct evidence presented in our study that SMPs are a distinct subset of muscle satellite cells. We showed that most SMPs do express the canonical satellite cell marker Pax7, but that SMP markers (β1-integrin and CXCR4) are expressed by only ~80% of Pax7$^+$ cells. Thus, we conclude that SMPs are a subpopulation of satellite cells, which unlike Pax3-GFP$^+$ cells (Montarras et al., 2005) do not require specialized transgenic mouse strains for their isolation. More importantly, however, our work rigorously demonstrates distinctive functional and physiological properties of SMPs (Cerletti et al., 2008; Sherwood et al., 2004), an essential step in the effective characterization of any stem or progenitor cell population (Wagers and Weissman, 2004). In particular, we have shown that SMPs are the only subset of myofiber-associated cells that exhibits clonal myogenesis in vitro and a robust myogenic contribution in vivo. Our more recent experiments using intramuscular transplantation of single GFP$^+$ SMPs further demonstrate that ~50% of muscles transplanted with a single SMP exhibit detectable myofiber engraftment (n = 10 muscles, M.C. and A.W., unpublished data). Given that previous studies indicate that 99% of myogenic donor cells perish almost immediately upon transplant (Beauchamp et al., 1999), these data clearly indicate that selection for SMP markers yields a unique, highly purified population of cells that is well suited to in vivo cell therapy.

The second concern raised by Dr. Partridge relates to our conclusion that high-level in vivo engraftment of myofibers by donor SMPs results in improved physiological function in recipient muscles. His challenge is based largely on the fact that we presented our data in a chart that correlated muscle engraftment level with the fold difference in contractile activity of SMP-treated versus mock-treated contralateral muscles in the same animal (see Figure 5, Cerletti et al., 2008). We maintain that this is an appropriate method of analysis because the comparison of SMP-treated versus mock-treated muscles in the same mouse accounts for variability in dystrophic disease and in engraftment efficiency in individual *mdx* recipients. In fact, clinical trials for myoblast transplantation and for exon skipping use similar comparisons to assess treatment efficacy (http://www.clinicaltrials.gov/, reviewed in Cossu and Sampaolesi, 2007). In any case, we also provided the same data in raw form in the Supplemental Data of our paper (Table S1).

Using the data in our Table S1 (Cerletti et al., 2008), Dr. Partridge has reanalyzed our results and comes to the conclusion that the only reason our SMP-treated muscles showed improvement is that they were compared to contralateral muscles that were particularly weak. This conclusion is based on replotting of our data using a linear regression model and on parametric correlation tests, which Dr. Partridge states "fall short of statistical significance at 5%" (in fact, the actual values are $p = 0.055$ and $p = 0.056$, using Pearson's product-moment correlation, for the specific peak force and the integrated area under the curve, respec-

tively). However, as discussed below, Dr. Partridge's use of linear regression and parametric statistics to re-evaluate our data is incorrect.

To address the concerns raised by Dr. Partridge in his Correspondence, we submitted our raw data to statistics experts in the Department of Biostatistics and Computational Biology at the Dana-Farber Cancer Institute, and they performed an independent analysis. This analysis determined that the data are not adequately represented by the linear regression model that Dr. Partridge uses. The linear regression model requires that the data being analyzed show a linear relationship and a normal distribution; yet our data meet neither of these requirements. Instead, our data are more appropriately represented by a nonlinear smoothing Lowess fit (locally weighted polynomial regression) (Cleveland, 1979; Cleveland and Devlin, 1988), shown here in Figure S1 available online. In addition, because the data are not normally distributed and have a small sample size, they should be analyzed using rank-based nonparametric statistics, as in Figure S1 shown here, to more robustly assess correlation and differences between groups.

In Figure S1, we replot our raw data (Table S1, Cerletti et al., 2008), showing the average change in peak force and integrated area under the curve (AUC), and separately analyze the SMP-treated (red triangles) and mock-treated (black diamonds) animals. This analysis confirms a clear and significant correlation between the level of engraftment by GFP^+ SMPs and improvement in contractile force of the SMP-treated muscles (Figures S1A and S1B; $p = 0.01$ for each plot by Spearman's rank test). Contrary to Dr. Partridge's assertion, this correlation is not dependent on the strength of the mock-transplanted contralateral muscles, which do not show a correlation with engraftment rate ($p = 0.17$ and $p = 0.14$ by Spearman's rank test for peak force or AUC, respectively).

In a further test of Dr. Partridge's claim, we also performed subset analysis, comparing data from animals that showed significant functional improvement in their engrafted muscles (i.e., animals exhibiting a difference in specific peak force of >1-fold when comparing their SMP-treated and mock-treated muscles) to data obtained from analysis of a separate cohort of untreated *mdx* mice. We found no significant difference in peak force production between the mock-treated muscles (n = 10, median = 12.4, range 1.6–39.1 mN/mm^2) and untreated *mdx* control mice (n = 9, median = 5.4, range 2.4–28.9 mN/mm^2; $p = 0.11$ by two-sided Wilcoxon rank-sum exact test). Thus, in our view, Dr. Partridge is incorrect in his contention that the contralateral mock-treated muscles of the highly engrafted animals in our study were exceptionally weak and that the strength improvements we observed in highly engrafted muscles arose solely as a result of this weakness.

Furthermore, consistent with the conclusions of our publication, this subset analysis also confirmed that the SMP-engrafted muscles in our study exhibited greater force production than both the contralateral mock-treated muscles and the untreated muscles of control *mdx* mice. In particular, muscles from SMP-engrafted mice showed significantly greater force production (n = 10, median = 30, range 1.9–43.0 mN/mm^2) than untreated *mdx* muscles (see above, $p = 0.004$ by two-sided Wilcoxon rank-sum exact test). In addition, for SMP-treated muscles versus mock-treated muscles (n = 10), the median fold change in average peak force was 1.8 (range, 1.1- to 5.5-fold) and was significantly greater than 1 ($p = 0.009$) by a one-sided Student's t test. As highlighted in the Correspondence by Dr. Partridge, one highly engrafted animal did show a surprisingly large difference in force generation (~5-fold greater); however, we note that the median difference in force production between SMP-engrafted and mock-treated muscles in this group of animals was more modest (1.8-fold).

In conclusion, although we appreciate the close consideration of our paper by Dr. Partridge, we show here that his reanalysis of our data is not based on proper statistical methods and therefore does not in fact challenge the original interpretations of our paper. The points he raises do not alter the main conclusions of our work, namely, that FACS-purified SMPs are a prospectively isolatable subset of canonically defined muscle satellite cells and exhibit robust engraftment to generate both functional muscle fibers and undifferentiated satellite cells upon intramuscular transplant. These properties suggest that SMPs represent a therapeutically promising stem cell population for immediate repair of dystrophin-deficient muscle as well as an enduring source of regenerative cells that can be recruited in response to future muscle injuries.

Supplemental Data

Supplemental Data include one figure and can be found with this article online at http://www.cell.com/supplemental/S0092-8674(08)01510-9.

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